Data Summary and Review on the Acute Toxicity of AE C656948 SC 500A G (Fluopyram formulation) to Freshwater Invertebrates - *Daphnia* sp.

PMRA Submission Number N/A EPA MRID Number 47372325

Data Requirement:	PMRA Data Code	9.3.2
	EPA DP Barcode	353315
	OECD Data Point	{……}
	EPA MRID	47372325
	EPA Guideline	850.1010; 72-2

Test material: **AE C656948 SC 500A G** **Purity:** 41.5% w/w (504.0 g/L)
Common name: Fluopyram
Chemical name: IUPAC: N-{2-[3-chloro-5-(trifluoromethyl)pyridine-2-yl]ethyl}-2-(trifluoromethyl)benzamide
CAS name: Not reported
CAS No.: $C_{16}H_{11}ClF_6N_2O$
Synonyms: 102000016460 (Specification number)

Reference/Submission No.: {.....}

Company Code	{.....}	[For PMRA]
Active Code	{.....}	[For PMRA]
Use Site Category:	{.....}	[For PMRA]
EPA PC Code	080302	

CITATION: Bruns, E. 2007. Acute Toxicity of AE C656948 SC 500A G to the Waterflea *Daphnia magna* in a Static Laboratory Test System. Unpublished study performed by Bayer CropScience AG (Development, Ecotoxicology), Monheim, Germany. Laboratory report number EBGMP067; GLP study identification number E 320 3235-8. Study sponsored by Bayer CropScience AG. Study completed on October 26, 2007.

EXECUTIVE SUMMARY:

The 48-hour acute toxicity of AE C656948 SC 500A G to *Daphnia magna* was studied under static conditions. Daphnids were exposed to nominal concentrations of 0 (negative control), 3.95, 7.11, 12.8, 23.1 and 41.5 mg a.i./L; mean-measured concentrations were <0.10, 3.80, 6.48, 11.6, 20.4 and 38.2 mg a.i./L. Mortality and sublethal effects were assessed daily. The 48-hour EC_{50} and NOAEC values, based on immobility, were >38.2 and 11.6 mg a.i./L, respectively. The sublethal effect included immobility.

Based on the results of this study, AE C656948 SC 500A G would be classified as practically nontoxic to *Daphnia magna* on an acute toxicity basis up to the limit of water solubility for Fluopyram, in accordance with the classification system of the U.S. EPA.

This study is classified as [scientifically sound or unsound] and {does or does not} satisfy guideline requirements for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age (e.g., 1st instar): 1st instar (<24 hours old)
Test Type (Flow-through, Static, Static Renewal): Static

NOAEC: 11.6 mg a.i./L Probit Slope: N/A
EC_{50}: >38.2 mg a.i./L
Endpoint(s) Affected: Immobilization

I. REPORTED MATERIALS AND METHODS

GUIDELINE FOLLOWED: The method used in this study followed the recommendations of the U.S. EPA FIFRA § 72(2); OECD Guideline 202 (2004); U.S. EPA Pesticide Assessment Guidelines, Subdivision E, § 72-2 (1982); EEC Directive 92/69/EEC and 92/69/EWG, part C.2 (1992); EU Council Directive 91/414/EEC (1991); Canadian PMRA Ref.: DACO 9.3.2; OPPTS 850.1010 (1996); and JMAFF 12 Nousan No. 8147 (2000).

COMPLIANCE: Signed and dated No Data Confidentiality, GLP and Quality Assurance statements were provided. This study was conducted in compliance with the current OECD Principles of Good Laboratory Practice (GLP) and with the current Principles of Good Laboratory Practice according to Annex 1 of the German chemical law (ChemG), dated June 20, 2002 [except for the screening work for contaminants in the dilution water]; and FIFRA § 10(d)(1)(A), (B) or (C). The testing facility "BCS-Development, Ecotoxicology" has been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (GLP certificate reference: II A 5 – 31.11.60.05, August 31, 2007). The analytical test site "BCS-Development, Residues, Operator and Consumer Safety" has been inspected and certified as working in compliance with the Principles of Good Laboratory Practice by the competent authorities (GLP certificate reference: II A 5 – 31.11.91.02, August 31, 2007).

A. REPORTED MATERIALS:

1. Test material AE C656948 SC 500A G

Description: Light brown fluid

Lot No./Batch No. : 2006-008200

Purity: 41.5% w/w (504.0 g/L)

Stability of compound under test conditions: Chemical analysis of freshly prepared solutions at test initiation revealed concentrations between 83-95% (mean 90%) of nominal. Chemical analysis of aged test solutions at the end of the 48-hour exposure period ranged from 92-98% (mean 94%) of nominal and 101-103% of 0-hour concentrations, demonstrating stability in the test system.

Storage conditions of test chemicals: 25±5°C (+2 to +30°C also reportedly acceptable).

Physicochemical properties of AE C656948 SC 500A G.

Parameter	Values	Comments
Water solubility at 20EC	Not reported	

Parameter	Values	Comments
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

2. Test organism:

Species: *Daphnia magna*

Age at test initiation: 1st instar (<24 hours old)

Source: Bayer Laboratory stock breeding; reportedly descended from at least the fourth (or later) brood of coeval parent daphnids (20-28 days ± 12 hours old)

B. REPORTED STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A non-GLP preliminary assay (range-finder) was reportedly conducted to estimate the approximate toxicity level of the test item. The definitive exposure concentrations were based on the results of the range-finding assay. Further details not reported.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details
Acclimation	
Period:	<24 hours
Conditions: (same as test or not)	Same
Feeding:	Three times per week with living cells of the green alga *Desmodesmus subspicatus*; daphnids reportedly not fed during study
Health: (any mortality observed)	No males, ephippia or dead animals were reportedly present in the cultures
Duration of the test	48 hours
Test condition	
Static/flow-through	Static
Type of dilution system for flow-through method.	N/A
Renewal rate for static renewal	N/A
Aeration, if any	None
Test vessel	
Material: *(glass/stainless steel)* Size: Fill volume:	Glass beakers 100 ml 50 ml
Source of dilution water	Fully defined, artificial water (type "Elendt M7").

Parameter	Details
Water parameters	
Hardness	14 German degrees (=249 mg/L $CaCO_3$)
pH	8.0-8.1
Dissolved oxygen	8.0-8.7 mg/L
Temperature	18-22°C
Total Organic Carbon	<2 mg/L
Particulate matter	<5 mg/L
Metals	Not detected (<0.1 or <1 µg/L)
Pesticides	Not detected (<0.01 or 0.05 µg/L)
Chlorine	<0.01 mg/L
Number of replicates	
Negative control:	6
Treatments:	6

Parameter	Details
Number of organisms per replicate Negative control: Treatments:	 5 5
Treatment concentrations Nominal: Mean-measured:	 0 (negative control), 3.95, 7.11, 12.8, 23.1 and 41.5 mg a.i./L <0.10, 3.80, 6.48, 11.6, 20.4 and 38.2 mg a.i./L
Solvent (type, percentage, if used)	None used
Lighting	"Cool white" fluorescent bulbs were reportedly used in an 18:8 hours light cycle, at a light intensity of max. 1200 lux
Stability of chemical in the test system	Stable
Recovery of chemical Level of Quantitation Level of Detection	 5 µg/L 1.7 µg/L
Positive control {if used, indicate the chemical and concentrations}	N/A
Other parameters, if any	N/A

2. Observations:

Table 2: Observations

Criteria	Details

Parameters measured including the sublethal effects	Immobilization
Observation intervals	24 and 48 hours
Were raw data included?	Yes
Other observations, if any	N/A

II. REPORTED RESULTS:

A. REPORTED MORTALITY:

Mortality was not included in the study report.

Table 3: Effect of AE C656948 SC 500A G on Mortality of *Daphnia* sp.*

Treatment (mg a i./L) Mean-Measured and (Nominal)	No. of organisms	Observation period					
		Day x		Day x		Day x	
		No Dead	% mortality	No Dead	% mortality	No Dead	% mortality
Negative Control	N/A	N/A	N/A	N/A	N/A	N/A	N/A
3.80 (3.95)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
6.48 (7.11)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
11.6 (12.8)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
20.4 (23.1)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
38.2 (41.5)	N/A	N/A	N/A	N/A	N/A	N/A	N/A
NOAEC	N/A	N/A	N/A	N/A	N/A	N/A	N/A
LC_{50}	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Positive control, if used Mortality: LC_{50} NOAEC:	N/A						

*Mortality was not included in the study report

B. REPORTED SUBLETHAL TOXICITY ENDPOINTS:

No sublethal effects were reportedly observed among daphnids in the negative control group, or among animals in the 3.80, 6.48 or 11.6 mg a.i./L treatment groups. Thirteen percent of animals exposed to 20.4 mg a.i./L of AE C656948 SC 500A G experienced immobility. Immobility was also reportedly observed among 20% of daphnids in the 38.2 mg a.i./L treatment group.

Table 4: Effect of AE C656948 SC 500A G on Immobilization - *Daphnia* sp.

Treatment (mg a.i./L) Mean-Measured and (Nominal)	Observation period			
	24 Hours		48 Hours	
	end-point	% affected	end-point	% affected
Negative Control	Immobilization	0	Immobilization	0
3.80 (3.95)	Immobilization	0	Immobilization	0
6.48 (7.11)	Immobilization	0	Immobilization	0
11.6 (12.8)	Immobilization	0	Immobilization	0
20.4 (23.1)	Immobilization	0	Immobilization	13
38.2 (41.5)	Immobilization	0	Immobilization	20
NOAEC	11.6 mg a.i./L			
LOAEC	20.4 mg a.i./L			
EC_{50}	>38.2 mg a.i./L			
Positive control, if used % sublethal effect: EC_{50}	N/A			

C. REPORTED STATISTICS:

The study author did not conduct statistics to determine an EC_{50} because the highest test concentration (100 mg form/L) caused only 20% immobilization.

III. REVIEWER'S EVALUATION

A. DEVIATIONS FROM GUIDELINES:

1. No transition-period was integrated into the light-dark cycle.
2. The fill volume (100 ml) was less than the minimum EPA recommended fill volume of 200 ml.
3. Hardness measured during the definitive toxicity test (249 mg/L $CaCO_3$) was higher than the maximum recommended hardness level of 180 mg/L $CaCO_3$.

B. OTHER STUDY DEFICIENCIES: None.

C. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Due to less than 50% immobilization among daphnids at any one treatment level, the reviewer did not perform statistical analysis to determine the 48-hour EC_{50} value. Therefore, the EC_{50} and NOAEC values were visually determined; the reviewer calculated mean-measured concentrations and they are reported in the Executive Summary and Results sections of this DER (See Appendix I).

NOAEC: 11.6 mg a.i./L
EC_{50}: >38.2 mg a.i./L 95% C.I.: N/A
Probit Slope: N/A 95% C.I.: N/A

D. ADDITIONAL REVIEWER COMMENTS:

The study author reported that "the clone of the primary *Daphnia magna* culture used in the study were classified as genotype No. 2 by Dr. Bradley, University of Sheffield, Department of Zoology (report of February 3, 1988). This clone was later renamed as "type B" according to BAIRD, D.J. et al. (1991)."

The in-life portion of the definitive toxicity test was conducted from May 21-24, 2007.

E. CONCLUSIONS:

This study is/is not scientifically sound and is thus acceptable/unacceptable. The 48-hour EC_{50} and NOAEC values were >38.2 and 11.6 mg a.i./L, respectively, based on immobility. Based on the results of this study, AE C656948 SC 500A G would be classified as practically nontoxic to *Daphnia magna* on an acute toxicity basis up to the limit of water solubility for Fluopyram, in accordance with the classification system of the U.S. EPA.

Results Synopsis

NOAEC: 11.6 mg a.i./L Probit Slope: N/A
EC_{50}: >38.2 mg a.i./L
Endpoint(s) Affected: Immobility

IV. REFERENCES:

Baird, D.J. et al. (1991) A Comparison Study of Genotype Sensitivity to Acute Toxic Stress Using Clones of *Daphnia magna* STRAUS. Ecotoxicological and Environmental Safety 21, 257-265.

Elendt, B.P. and Bias, W.R. (1990) Trace Nutrient Deficiency in *Daphnia magna* Cultured in Standard Medium for Toxicity Testing. Effects of the Optimisation of Culture Conditions on Life History Parameters of *D. magna*. Water Research 24 (9), 1157-1167.

Bruns, E. (2007) Acute Toxicity of Potassium Dichromate (p.a. grade) to the Waterflea *Daphnia magna*. Bayer AG unpublished report ID.: Reference 01/2007.

OECD Guideline No. 202 (Guideline for Testing of Chemicals, "*Daphnia* sp., Acute Immobilisation Test, adopted April 13, 2004).

EEC Directive 92/69/EWG, part C.2 (1992).

Japanese MAFF guideline (JMAFF 12 Nousan No. 8147 (2000)).

EPA FIFRA Guideline 72-2 "Acute Toxicity Test for Freshwater Invertebrates".

OPPTS Series 850.1010 (1996).

APPENDIX I: REVIEWER'S CALCULATION MEAN-MEASURED CONCENTRATIONS

nominal concentration	0-hour mean-measured	48-hour mean-measured	mean-measured	% of nominal	% of 0-hour
3.95	3.73	3.86	3.795	96.1	103.5
7.11	6.41	6.55	6.48	91.1	102.2
12.8	11.5	11.6	11.55	90.2	100.9
23.1	19.3	21.6	20.45	88.5	111.9
41.5	37.8	38.6	38.2	92.0	102.1